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NONLETHAL RODENT REPELLENTS: DIFFERENCES IN CHEMICAL STRUCTURE AND EFFICACY FROM NONLETHAL BIRD REPELLENT

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Abstract—At least some anthranilates (e.g., methyl anthranilate), and acetophenones (e.g., orthoaminoacetophenone) are aversive to mice as well as to birds. Here we systematically examined nine acetophenone isomers (ortho, meta, para) and moieties (amino, hydroxy, methoxy) previously tested as drinking and feeding repellents for European starlings (*Sturnus vulgaris*). All nine substances reduced intake by mice in single-bottle tests. When molecular characteristics were examined, amino group reactivity and, to a lesser extent, isomeric position (i.e., resonance), were related to the strength of the avoidance response. Unlike effective avian repellents, the presence of intramolecular hydrogen bonds did not appear to affect avoidance responding.

Key Words—Acetophenone, anthranilate, chemosensory, mouse, *Mus musculus*, repellent.

INTRODUCTION

Few nonlethal chemicals are available for rodent control, although they would be desirable in situations where lethal control poses health or aesthetic risks. Those substances that are available are either indiscriminately offensive, such

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as capsaicin (Meehan, 1988), or unpredictable in performance, such as denatonium benzoate, which varies inter- and intraspecifically (Beauchamp and Mason, 1991).

A promising strategy for the development of new rodent repellents may be molecular modeling where chemical structure is related to biological activity. Using this approach, we recently tested 36 derivatives of benzoic acids as bird repellents (Mason et al., 1991; Clark and Shah, 1991; Clark et al., 1991). Acetophenones are structurally similar to anthranilates, and both groups contain effective avian repellents. Three molecular features contribute to repellency: (1) the basicity of a substituted phenyl ring, (2) the presence of an electron-donating group in resonance with an electron withdrawing group on a phenyl ring, and (3) a heterocyclic ring in the same pi cloud as the phenyl ring, with the ring comprised of an intramolecular hydrogen bond or covalently bonded heteroatoms (Clark and Shah, 1991).

At least one anthranilate (methyl anthranilate), and one acetophenone (orthoaminoacetophenone) are aversive to mice (Nolte et al., 1993) as well as to birds (Mason et al., 1991). Here, we examined the repellency of additional acetophenone isomers (ortho, meta, para) and moieties (amino, hydroxy, methoxy) to clarify the effects of isometric changes on repellency (viz., Clark and Shah, 1991). We studied amino, methoxy and hydroxy substitutions because these configurations allowed a systematic test for the effects of basicity, position (isomerization), and hydrogen bonding on repellency.

METHODS AND MATERIALS

Subjects. Two hundred sixteen experimentally naive 30- to 35-day-old male mice (*Mus musculus*) were individually caged (27 × 21 × 14 cm) under a 12:12 light–dark cycle (light onset at 0700 hr) at 23°C and given free access to 8604-00 Wayne Rodent Blox. Prior to testing, the animals were allowed free access to tap water presented in 10-ml syringes fitted with sipper tubes. These same tubes were used during the experimental procedures described below.

Chemicals. Orthoaminoacetophenone (CAS #551-93-9), metaaminoacetophenone (CAS #99-03-6), paraaminoacetophenone (CAS #99-92-3), orthomethoxyacetophenone (CAS #579-74-8), metamethoxyacetophenone (CAS #586-37-8), paramethoxyacetophenone (CAS #100-06-1), orthohydroxyacetophenone (CAS #118-93-4), metahydroxyacetophenone (CAS #121-71-1), and parahydroxyacetophenone (CAS #99-93-4) were obtained from Aldrich Chemical Company (Milwaukee, Wisconsin). Each moiety had a phenyl ring with an electron-donating primary amino, hydroxy, or methoxy group and an electron-withdrawing carbonyl group. The strength of donation was ranked as: amino > methoxy > hydroxy. Isomers of each moiety differed only in their substitution patterns on the phenyl ring (Figure 1).

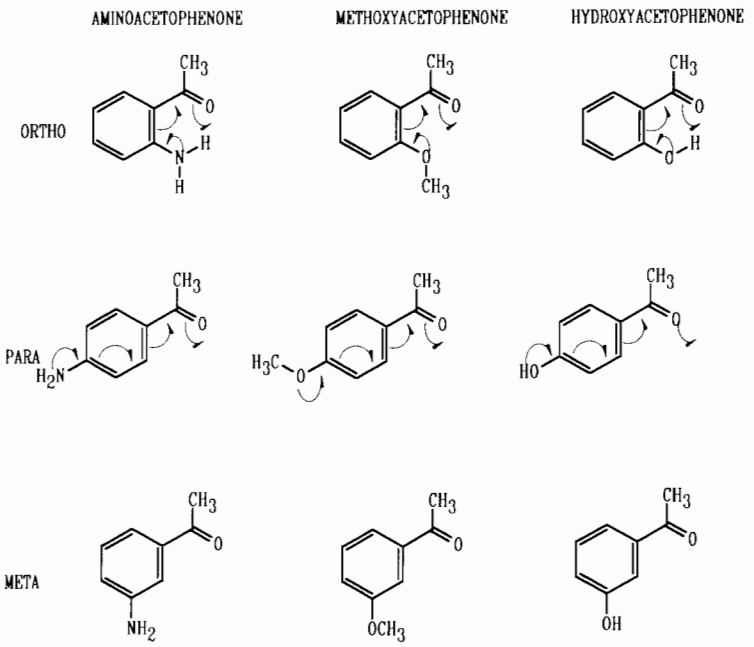

FIG. 1. Molecular structure of the isomers of amino-, methoxy- and hydroxyacetophenones. The arrows indicate donation paths of lone pairs of electrons.

Because acetophenones are generally insoluble in water, we mixed each compound in water under low heat to yield saturated emulsions with concentrations at 1.0% (w/w). Lower concentrations of 0.5% and 0.25% were prepared by serial dilution.

Procedure. Mice were randomly assigned to 27 treatment groups (N = 8/group), and adapted to an 18-hr (1500–0900 hr) water-deprivation schedule. Adaptation was followed immediately by four days of pretreatment. On each pretreatment day, tap water was presented at 0900 hr, and water intake between 0900 and 1200 hr was determined to the nearest 0.1 ml. Between 1200 and 1500 hr, animals were provided ad libitum access to tap water.

A four-day treatment period immediately followed pretreatment. Treatment procedures were identical to those described for pretreatment, except that each group was given a different chemical and concentration during the 3-hr measurement period.

Analysis. Prior to treatment, we tested whether water intake among the 27 groups was equal using a one-way repeated-measures analysis of variance (ANOVA). Equality of water intake among groups was a precondition for further

testing, allowing an unbiased estimate of concentration, chemical, and pre- vs. treatment differences in subsequent analyses (Games, 1979).

We tested for differences among experimental factors using a four-way repeated-measures ANOVA. Fluid intake was the dependent variable. Between-subjects factors were chemical (nine levels) and concentration–group (three levels). It is important to bear in mind that mice received water only during the pretreatment period and chemicals only during the treatment period, thus concentration effects were confounded with group effects. However, since the qualifying one-way analysis of variance described above indicated no pretreatment differences for intake among groups, any observed effects were reasoned to be due to chemical concentration effects. Within-subjects (i.e., repeated) factors were period (two levels, pre- vs. treatment period) and day (four levels).

Because four-factor analyses of an experiment are often difficult to interpret, we used Duncan's multiple-range tests to identify post-hoc differences among means for specific structured comparisons of interest (e.g., chemical effects during the treatment period) (von Eye, 1990).

RESULTS

Pretest Fluid Intake Criterion. There was no interaction between days and groups, indicating that all groups of mice had the same fluid intake pattern across days ($P = 0.301$). Furthermore, there were no day effects ($P = 0.352$). More importantly, the lack of a group effect ($P = 0.805$) indicated that assignment of groups to chemical and concentration treatment could proceed without biasing further analyses.

Overall Four-Factor Effects. All but three of the main and interaction effects of the four-way repeated measures ANOVA were significant (Table 1). Because there were no group effects during the pretest period (above), all period × day × within-subjects and period × within-subjects effects were assumed to be related to aversion of chemically treated water. This aversion is illustrated by inspection of the period × concentration × chemical-group profiles ($P < 0.001$). Furthermore, aversions were more pronounced at higher concentrations ($P < 0.001$).

Factors involving day effects were generally significant. Fluid intake during the treatment period tended to decrease as a function of time ($P < 0.001$; Table 1). While day interactions with chemical or concentration group were also significant, there was no systematic pattern for fluid intake that could be attributed to biological or chemical effects.

There were differences among chemicals ($P < 0.001$). Post-hoc range tests indicated that the aminoacetophenones, the most basic of the compounds considered, were also the most repellent (Figure 2). The remainder of chemicals

TABLE 1. *F* AND *P* VALUES FOR THE FOUR-WAY REPEATED MEASURES ANOVA

	df	MS	*F*	*P*
Between-subjects effects				
Constant	1	21371	5144.98	<0.001
Chemical	8	49.63	11.96	<0.001
Concentration group (CG)	2	139.12	33.99	<0.001
Chemical × CG	16	4.87	1.17	= 0.293
Error	188	4.15		
Period × within-subjects effects				
Period	1	2686.46	1809.31	<0.001
Chemical × period	8	50.13	33.76	<0.001
CG × period	2	134.48	90.57	<0.001
Chemical × CG × period	16	3.85	2.60	<0.001
Error	188	1.48		
Day × within-subjects effects				
Day	3	1.46	5.25	<0.001
Chemical × day	24	.78	2.80	<0.001
CG × day	6	.53	1.92	= 0.076
Chemical × CG × day	48	.43	1.54	= 0.013
Error	564	.28		
Period × day × within subjects effects				
Period × day	3	3.11	9.68	<0.001
Chemical × period × day	24	0.83	2.53	<0.001
CG × period × day	6	0.24	0.76	= 0.603
Chemical × CG × period × day	48	0.53	1.65	<0.005
Error	564	0.32		

yielded a graded aversion response. Ortho isomers of both methoxy and hydroxy (less basic) functions tended to be more repellent than the meta isomers. Para isomers were intermediately effective repellents with respect to ortho- and meta isomers of the acidic functions.

The four-way repeated measures ANOVA indicated a significant chemical × period × concentration × day effect ($P < 0.001$). Post-hoc tests were undertaken for all concentration groups for each chemical to elucidate the nature of the period and day effect.

Aminoacetophenones. Post-hoc tests indicated that fluid intake during the treatment period was reduced relative to the pretreatment period for all aminoacetophenone groups (Figure 3A–C). Fluid intake was similar across the four-day treatment period for all concentration groups of orthoaminoacetophenone (Figure 3A). Fluid intake decreased during the treatment period for all concentration groups of paraaminoacetophenone (Figure 3B) and all concentration groups of metaaminoacetophenone (Figure 3C). There was no indication that

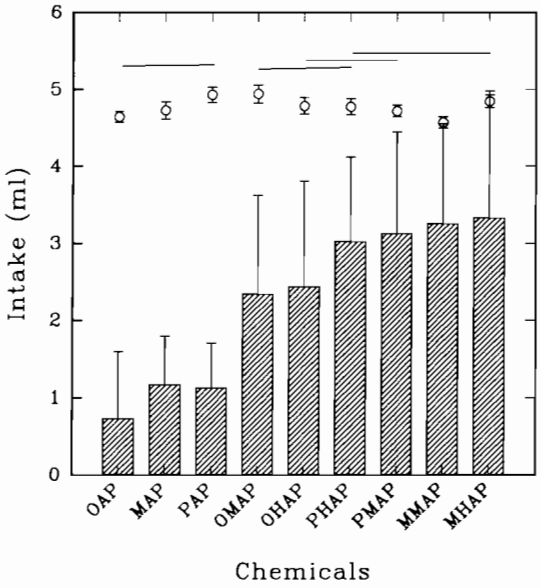

FIG. 2. The mean fluid intake by mice during the treatment period (hatched bars) for each of the test chemicals. Values were averaged over all concentration groups. The horizontal lines depict homogeneous subsets as determined by Duncan's multiple-range tests. The open circles depict the mean pretreatment water ingestion by mice given each of the chemicals. Vertical capped bars depict 1 SE.

habituation or sensitization towards any of these repellents occurred, even after exposure for four days.

Methoxyacetophenones. Fluid ingestion decreased during the treatment period relative to the pretreatment period for the 1.0% and 0.5% concentration groups of orthomethoxyacetophenone (Figure 3D). There was no evidence of habituation or sensitization towards the repellent after four days. The repellent effect was weak for the 0.25% concentration group (Figure 3D).

Fluid intake was similar across all pretreatment and treatment days for the lowest concentration-group (0.25%) of paramethoxyacetophenone (Figure 3E). There were clear reductions, however, during the treatment period for the two higher concentration groups (Figure 3E). Again, there was no evidence of habituation or sensitization during the four-day treatment period.

There was no repellent effect for metamethoxyacetophenone for the 0.25% concentration group (Figure 3F). Intake was less during the treatment period than during the pretreatment period for the 0.5% concentration group (Figure 3F). Ingestion across days within each of the periods was similar. A similar trend was seen for the 1.0% concentration group (Figure 3F). As before, there

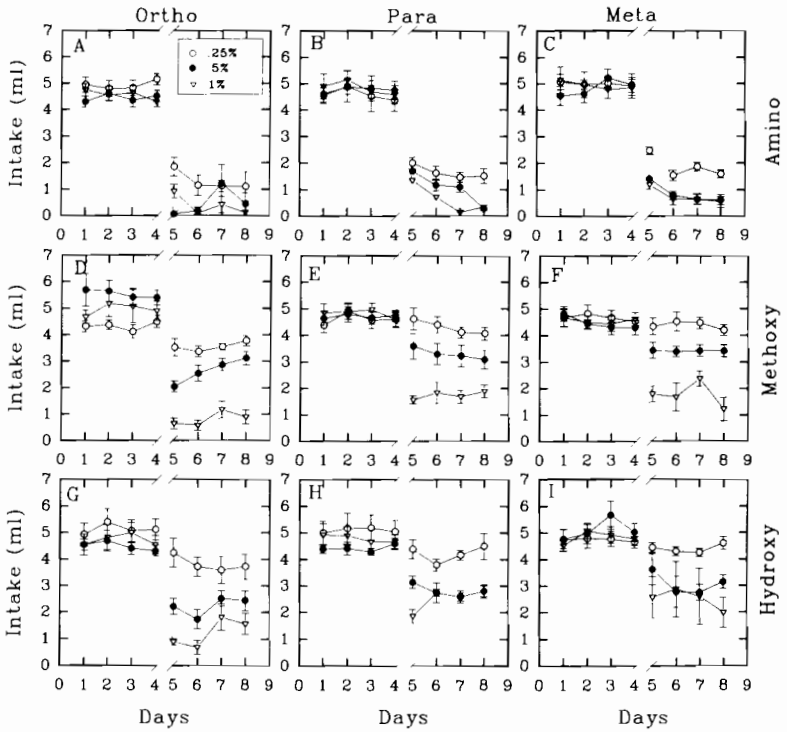

FIG. 3. Profiles of fluid ingestion by mice for the four-way interaction of chemical × day × period × concentration group. The pretreatment period consisted of days 1–4. The treatment period consisted of days 5–8. The break in the *x* axis represents the beginning of the treatment period. The vertical capped bars depict 1 SE.

was no evidence of an enhanced avoidance response across days as a function of time.

Hydroxyacetophenones. Fluid ingestion for orthohydroxyacetophenone decreased during the treatment period for the 1.0% and 0.5% concentration groups (Figure 3G). There was no evidence of habituation or sensitization towards these repellents after four days. Post-hoc tests showed that there was no significant difference in ingestion over time for the 0.25% concentration group.

Fluid ingestion for parahydroxyacetophenone decreased during the treatment period relative to the pretreatment period for the 1.0% and 0.5% concentration groups (Figure 3H). There was no indication of reduced repellency after four days of exposure to the repellent. At the lower concentration of 0.25%, there was no indication of a repellent effect (Figure 3H).

Fluid intake for metamethoxyacetophenone tended to be lower for the 1.0% and 0.5% concentration groups during the treatment period, although the level of ingestion on some treatment days was similar to pretreatment days (Figure 3I). There was no such ambiguity, however, for the 0.25% concentration group. At this concentration, there was no repellent effect.

DISCUSSION

In previous experiments with avian repellents (Clark and Shah, 1991; Clark et al., 1991; Mason et al., 1991), the strength of repellency decreased as a function of the positional isomer; ortho isomers were better repellents than para isomers, and these, in turn, were better than meta isomers, suggesting that electron donation by resonance was an important feature of repellency. Basicity (and/or electron-donating ability of the substituent group) was also important. Thus, amino substituents were both the most basic and the strongest repellent, whereas hydroxy substituents were the least basic and the weakest repellents. Finally, intramolecular hydrogen-bonding capacities were associated with repellency, although such bonding was not necessary for strong repellency to occur.

In this study we found that amino substituents were more repellent to mice than either methoxy or hydroxy substituents. Unlike with birds, however, we found no strong differences in repellency between methoxy and hydroxy substituents. For these less basic substituents, resonance appeared to contribute to repellency. The relative ranking of fluid intake by mice was consistent with this notion (i.e., ortho < para < meta). This is in contrast to birds, where the relative contribution of basicity to repellency was more important that resonance.

It is clear that mice differ from the previously tested bird species in their sensitivity towards acetophenones. A comparison of the concentration–response functions for birds and mammals suggests that birds have a lower threshold for aversion to the compounds tested (Clark and Shah, 1991). In mammals, except for the amino substituent, repellent effects disappeared at concentrations of 0.25%. Thus, it may be possible that for the nonamino substituents, mammals are responding to a concentration effect rather than structural, positional, or electronic features of molecules, which might manifest themselves at lower concentrations. This interpretation may be favored because, even though intake of methoxy and hydroxy substituents was a function of resonance, the degree of overlap among the chemicals suggests that the contribution of resonance to repellency may have been weak. Thus, we conclude that amino reactivity rather than basicity per se is the primary feature relating to repellency in mammals.

Management Implications. While we are cautious about extrapolating from the laboratory to the field, the present results have clear practical implications. For example, granular agricultural chemicals are a hazard to rodents and birds

that unwittingly ingest them. An additive with both rodent and bird repellent capabilities would be useful, particularly since no such repellent is available at present (Beauchamp and Mason, 1991). Cowbirds (*Molothrus ater*) reduce their intake of pellets containing pesticides when methyl anthranilate is present (Mason et al., 1993).

The repellents tested here might also have application as additives to packaging, plastics, and fabrics where any rodent damage is undesirable. Avoidance of all the chemicals in the present experiment was strong on the first day of treatment, at least for the higher ($\geq 0.5\%$) concentration. This suggests that at least some of these chemicals may be useful in inhibiting damage caused by sampling or explorative gnawing of rodents.

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